

What is claimed is:

1. A method for identifying virulence determinants of a bacteria comprising:
introducing at least one mutation into the genome of a bacteria;
culturing the mutated bacteria in the presence of an antimicrobial agent that kills
growing but not non-growing bacteria;
5 selecting surviving bacteria;
testing the selected surviving bacteria for virulence;
selecting the non virulent bacteria;
sequencing genetic material from said selected non virulent bacteria;
determining the site of mutation;
and comparing the sequence at the mutated site to the corresponding wild type
sequence.
2. The method of claim 1 wherein said bacteria is a mycobacteria.
3. The method of claim 2, wherein said mycobacteria is a slow growing mycobacteria.
4. The method of claim 3, wherein said slow growing mycobacteria is *Mycobacterium*
paratuberculosis.
5. The method of claim 1, wherein said mutation is by insertion of a transposon.
6. The method of claim 1, wherein said mutation is a random mutation.
7. The method of claim 1, wherein said antimicrobial agent is a fluoroquinolone.
8. The method of claim 7, wherein said fluoroquinolone is Bay y 3118.
9. The method of claim 8, wherein said Bay y 3118 is used at a concentration of at least
0.015 µg/mL.

10. The method of claim 1, wherein said antimicrobial is D-cycloserine.
11. The method of claim 10, wherein said D-cycloserine is used at a concentration of at least 25.0 µg/mL.
12. The method of claim 1, wherein said mutated bacteria is cultured in an intracellular culture system.
13. The method of claim 12, wherein said intracellular culture system is a macrophage culture system.
14. A method for identifying virulence determinants in *Mycobacterium paratuberculosis* comprising:
 - introducing at least one random mutation into the genome of a *M. paratuberculosis* bacteria by introduction of a transposon;
 - 5 infecting macrophages with said mutated bacteria
 - culturing the macrophages containing said mutated bacteria in the presence of a fluoroquinolone or D-cycloserine;
 - selecting surviving bacteria;
 - testing the selected surviving bacteria for virulence in an animal;
 - 10 selecting the non virulent organisms;
 - sequencing genetic material from said selected non virulent bacteria;
 - determining the site of mutation; and
 - comparing the sequence at the mutated site to the corresponding wild type sequence.
15. A composition for immunizing an animal against bacterial infection comprising:
 - a pharmaceutically acceptable carrier, diluent or excipient;
 - and at least one non-virulent strain of bacteria produced by the process comprising:
 - introducing at least one mutation into the genome of a bacteria;
 - 5 culturing the mutated bacteria in the presence of an antimicrobial agent that kills growing but not non-growing bacteria;
 - selecting surviving bacteria;

testing the selected surviving bacteria for virulence;
and selecting the non-virulent strains.

16. The composition of claim 15, wherein said bacteria is a mycobacteria.
17. The composition of claim 16, wherein said bacteria is a slow growing mycobacteria.
18. The composition of claim 17, wherein said slow growing mycobacteria is *Mycobacterium paratuberculosis*.
19. The composition of claim 15, wherein said mutation is by insertion of a transposon.
20. The composition of claim 15, wherein said mutation is a random mutation.
21. The composition of claim 15, wherein said antimicrobial agent is a fluoroquinolone.
22. The composition of claim 21, wherein said fluoroquinolone is Bay y 3118.
23. The composition of claim 22, wherein said Bay y 3118 is used at a concentration between of at least 0.015 µg/mL.
24. The composition of claim 15, wherein said antimicrobial is D-cycloserine.
25. The composition of claim 24, wherein D-cycloserine is used at a concentration of at least 25 µg/mL.
26. The composition of claim 15, wherein said mutated bacteria is cultured in an intracellular culture system.
27. The composition of claim 26, wherein said intracellular culture system is a macrophage culture system.

28. A composition for immunizing an animal against *Mycobacterium paratuberculosis* comprising:
a pharmaceutically acceptable carrier, diluent or excipient;
and at least one non-virulent strain of *M. paratuberculosis* produced by the process
comprising:
introducing at least one random mutation into the genome of a strain of *M.*
paratuberculosis by insertion of a transposon;
infecting macrophages with the mutated strain;
culturing the infected macrophages in the presence of a fluoroquinolone or D-
cycloserine;
selecting surviving *M. paratuberculosis* organisms;
testing the selected surviving organisms for virulence in an animal; and
selecting the non-virulent strains.
29. A composition for immunizing an animal against a bacteria comprising:
a pharmaceutically acceptable carrier diluent or excipient;
and at least one bacterial virulence determinant, the determinant identified by a
process comprising:
introducing at least one mutation into the genome of a bacteria;
culturing the mutated bacteria in the presence of an antimicrobial agent that kills
growing but not non-growing bacteria;
selecting surviving bacteria;
testing the selected surviving bacteria for virulence;
selecting the non-virulent strains;
sequencing genetic material from the selected non-virulent bacteria to determine the
site of the mutation; and
identifying the virulence determinant based on the site of the mutation.
30. The composition of claim 29, wherein said bacteria is a mycobacteria.
31. The composition of claim 30, wherein said mycobacteria is a slow growing mycobacteria.

32. The composition of claim 31, wherein said slow growing mycobacteria is *Mycobacterium paratuberculosis*.
33. The composition of claim 29, wherein said mutation is by insertion of a transposon.
34. The composition of claim 29, wherein said mutation is a random mutation.
35. The composition of claim 29, wherein said antimicrobial agent is a fluoroquinolone.
36. The composition of claim 35, wherein said fluoroquinolone is Bay y 3118.
37. The composition of claim 36, wherein said Bay y 3118 is used at a concentration of at least 0.015 µg/mL.
38. The composition of claim 29, wherein the antimicrobial is D-cycloserine
39. The composition of claim 38, wherein said D-cycloserine is used at a concentration of at least 25 µg/mL.
40. The composition of claim 29, wherein said mutated bacteria is cultured in an intracellular culture system.
41. The composition of claim 40, wherein said intracellular culture system is a macrophage culture system.
42. A composition for immunizing an animal against *Mycobacterium paratuberculosis* comprising:
a pharmaceutically acceptable carrier diluent or excipient;
and at least one *Mycobacterium paratuberculosis* virulence determinant, the determinant identified by a process comprising;
introducing at least one mutation into the genome of a strain of *Mycobacterium paratuberculosis* by insertion of a transposon;

10 infecting macrophages with the mutated strain;
culturing the infected macrophages in the presence of a fluoroquinolone or D-
cycloserine;
selecting surviving bacteria;
testing the selected surviving bacteria for virulence in an animal;
selecting the non-virulent bacteria;
sequencing genetic material from the selected non-virulent bacteria to determine the
15 site of the mutation; and
determining the virulence determinant based on the site of the mutation.

43. A method for inducing an immune response in an animal against paratuberculosis comprising administering to an animal an immune response inducing amount of the composition of claim 15.
44. A method for inducing an immune response in an animal against paratuberculosis comprising administering to an animal an immune response inducing amount of the composition of claim 29.
45. A method for diagnosing infection by a bacteria comprising:
obtaining a sample from an animal and determining the presence or absence in the sample of a bacterial virulence determinant, said determinant identified by the process of claim 1.
46. The method of claim 45, wherein said bacteria is a mycobacteria.
47. The method of claim 46, wherein said bacteria is a slow growing mycobacteria.
48. The method of claim 47, wherein said slow growing mycobacteria is *Mycobacterium paratuberculosis*.
49. The method of claim 45, wherein said animal has previously been administered the composition of claim 15.

50. The method of claim 49, wherein the composition administered contains a mutated form of the bacterial determinant whose presence or absence is determined.
51. The method of claim 45, wherein said animal has previously been administered the composition of claim 29.
52. The method of claim 51, wherein the composition administered contains a mutated form of the bacterial determinant whose presence or absence is determined
53. The method of claim 45 wherein the presence or absence of said bacterial determinant is determined by nucleic acid hybridization, nucleic acid amplification, or immunological assay.